



TENNESSEE BUREAU OF INVESTIGATION

Forensic Services Division

Forensic Chemistry Standard Operating Procedure Manual

High Performance Liquid Chromatography

23.0 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

23.1 Application

High Performance Liquid Chromatography (HPLC) is a presumptive test for comparing retention times (RT) for unknown samples versus known working standards.

23.2 Equipment

The TBI FCU utilizes Hewlett Packard and Agilent Technologies high performance liquid chromatographs. Compounds of interest are separated using a variety of mobile phases and columns. These compounds are detected using an UV/Vis spectrophotometric detector. All systems are equipped with proprietary software for instrument control and data analysis.

23.3 Reagents and Standards

The mobile phase should be made with HPLC grade or better solvents.

A caffeine check standard will be used to verify instrument performance. Working standards of legally significant substances will be available for RT comparisons.

23.4 Method

Since solvent type can affect retention time, samples will be extracted into the same solvent system as the standards whenever possible. The prepared sample will be filtered to remove any particulate matter that could clog or damage the column.

Samples are introduced to the mobile phase using an auto-sampler with a sample loop. The sample loop is cleaned internally by the instrument. The operator should flush the column with the mobile phase solvents before and after use.

23.5 Quality Assurance

Quality control protocols for the HPLC follow the same procedure as outlined in the Gas Chromatography chapter (Section 21.1.5).

23.6 Performance Verification and Acceptance Criteria

The column is flushed with high purity reverse osmosis water, methanol, and heptane sulfonic acid. The detector is turned on, and the instrument response will be monitored to verify that the column is not eluting compounds during the flushing process.

A caffeine standard will be run before each use to ensure the instrument is functioning properly. Maintenance will be performed if the standard does not give a satisfactory result. The instrument will be removed from service, and the unit supervisor will be notified if it continues to give unsatisfactory results for the check standard.

Refer to the manufacturer's manual for maintenance guidelines.



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23.7 Interpretation

The sample's RT must fall within +/- 2% of the working standard's RT for the analyst to determine that it is "consistent with" the compound of interest.